INTRODUCTION

The United States Environmental Protection Agency (USEPA) estimates 40% of western US headwater streams are contaminated by hard rock mining (USEPA 2000). Though persistent impacts of mining to aquatic biota are widely documented, mine exploration and development continues to expand in the US and worldwide (Kemble et al. 1994, Pascoe et al. 1994, Farag et al. 1998, Barry et al. 2000, Maret and MacCoy 2002, USGS 2004, Wilburn 2005). Currently, aquatic life criteria regulating metals pollution associated with mine waste are defined as either acute or chronic based on dose-response relationships evaluated in a laboratory setting. Calculation of acute aquatic life criteria generally relies on lethal concentrations of pollutants over short periods of time (i.e., LC50 in less than 96 hours, EPA 2012). Chronic criteria are calculated using concentrations resulting in lethal and sublethal impacts to test species such as reduced growth and fecundity over longer periods of time (e.g., one tenth the lifespan of the test organism or longer, EPA 2012). Relying solely on acute and chronic aquatic life criteria risks overlooking indirect impacts of contaminant exposure which may alter behavior and ultimately lower survival (e.g., olfaction, osmoregulation). In order to maintain ecological integrity of aquatic ecosystems in light of expanding mine development, it is essential to consider acute, chronic, and indirect impacts from mining to ecologically influential and environmentally sensitive species (Carignan and Villard 2002).

Sculpin species are widespread throughout the northern hemisphere, and often dominate freshwater fish assemblages (Beauchamp 1990, Adams and Schmetterling 2007). Because of their high abundance and biomass in many freshwater ecosystems, sculpins are important predators of macroinvertebrates and fish eggs, and important prey to sport fishes and terrestrial wildlife (Poe et al. 1991, Foote and Brown 1998, Birzaks et al. 1999, Hodgens et al. 2004, Madenjian et al. 2005, Adams and Schmetterling 2007, Hauer et al. 2007). Sculpins are also less mobile than many fish taxa, and are often more sensitive to stream acidification and metals contamination than species typically used to establish water quality criteria (Matuszek et al. 1990, Maret and MacCoy 2002, Woodling et al. 2002, Gray et al. 2004, Brinkman and Woodling 2005, Dubé et al. 2005, Besser et al. 2007, Natsumeda 2007). Consequently, the overarching objective of this proposal is to evaluate the utility of sculpins as bioindicators for the purpose of measuring impacts of mining to aquatic ecosystems.

STUDY AREA

The majority of Alaska's lakes and streams are not impacted by urban or industrial development and have never been evaluated for baseline water quality or aquatic biota (ADFG 2012). However, Alaska is presently experiencing an unprecedented boom in mineral exploration statewide (Szumigala et al. 2011). Included in exploration leases are portions of the Bristol Bay watershed which supports the world's largest remaining sockeye salmon fishery (Ruggerone 2010, Schindler 2010, Figure 1). Salmon have sustained native communities in the region for millennia and are an important economic and food resource locally and globally (Krieg et al. 1998, Stickman et al. 2003, Krieg et al. 2005, Duffield et al. 2007, Fall et al. 2009).

Currently, the most advanced mineral exploration project is at one of the world's largest copper deposits, the Pebble deposit, which straddles two of Bristol Bay's most productive watersheds, the Nushagak and Kvichak drainages (Ruggerone et al. 2010, Ghaffari et al. 2011). In light of potential mine development, scientists have studied the surrounding ecosystem. Geochemical surveys indicate the deposit is sulfide-bearing, and that mining is likely to generate acid mine drainage producing elevated copper concentrations of area waters (NDM 2005,

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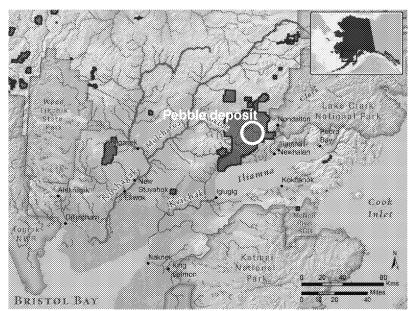


Figure 1. Region of mining claims in the Bristol Bay watershed, southwest Alaska. Mining claims are shaded in dark grey, and total more than $2000~\rm km^2$. The approximate location of the Pebble deposit, which straddles the Nushagak and Kvichak drainages, is encircled within the largest block of mine claims.

Blowes et al. 2007, PLP 2011). Biological surveys established that fish are present throughout streams potentially impacted by mining, with 75% of headwaters containing anadromous salmon species, and 96% of headwaters containing resident fishes (Woody and O'Neal 2010). Slimy sclupin (Cottus cognatus) are ubiquitous, occurring at over two times the density of other resident and anadromous fishes and composing the majority of the biomass in headwaters (Figure 2, O'Neal and Woody *In prep*). Fish are supported by exceptionally dilute waters with metals concentrations frequently below detection limits (Table 1).

Because of the low alkalinity of streams, pH will

drop easily if small amounts of acid drainage are released during the course of mining (Zamzow 2011). Low pH, in turn, dissolves metals such as copper unearthed during mine development. Further, low organic matter in headwaters draining the Pebble deposit limits assimilation of copper in regional headwaters, ultimately risking contamination to exceptional fisheries habitat (MacRae et al. 1999, Craven et al. *In prep*). Slight increases in background copper concentrations—below limits commonly used to set acute and chronic water quality standards—are potentially harmful to salmon and other aquatic life (Clements et al. 1990, Eisler 1998, Sandahl et al. 2007, Baldwin et al. 2011, McIntyre et al. 2012).

Acute and chronic water quality standards regulating copper and other discharge are commonly calculated using either hardness (ADEC 2008) or the US Environmental Protection Agency's Biotic Ligand Model (BLM) which is intended to account for site specific water chemistry (pH, hardness, and dissolved organic carbon) and better estimate the concentration of copper that is biologically available and thus potentially harmful to aquatic life (USEPA 2007). However, recent research using stream water from the Pebble site indicates that both hardness-based and BLM-calculated copper criteria may fail to protect salmonid species and more

Table 2. Water quality characteristics of streams in and around mining claims in Bristol Bay, Alaska in spring 2008-2010; n=54. Data is from Zamzow 2011.

Parameter	Median	Minimum	Maximum
pН	7.16	5.39	7.66
Alkalinity (as mg/L CaCO3)	21	1	47
Hardness (mg/L)	16	1	42
Conductivity (umhos/cm)	45	9	97
Dissolved Organic Carbon (mg/L)	2.4	1	6.1
Copper, total (ug/L)	0.22	0.04	5.60
Copper, dissolved (ug/L)	0.19	0.04	3.57

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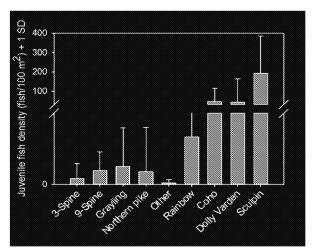


Figure 3. Density (fish per square meter) in headwater streams of the Nushagak and Kvichak Rivers. From O'Neal and Woody *In prep*.

sensitive sculpin in waters surrounding the Pebble deposit (Brinkman and Woodling 2005, Craven et al. *In prep.*). Given the high potential for acid mine drainage and increased copper concentrations associated with copper mining in the Nushagak and Kvichak watersheds, the dominance of relatively immobile sculpins in stream foodwebs, and their high sensitivity to copper and other metals, the goal of this proposed work is to evaluate the utility of slimy sculpin as bioindicators for the purpose of measuring aquatic ecosystem impacts associated with sulfide copper mining to provide a reference for future monitoring, and inform calculation of biologically meaningful water quality criteria in nearly pristine streams.

<u>APPROACH</u>: I propose to conduct research focused on slimy sculpin in headwater streams potentially impacted by sulfide-copper mining.

- Objective 1: Determine the utility of sculpins as bioindicators by estimating movement patterns.
- Objective 2: Estimate inter-annual population variability of sculpins within and outside the area of mine impact.
- Objective 3: Investigate acute, chronic, and indirect effects of copper to sculpins in site specific waters in both field and laboratory environments.

Objective 1: Is sculpin movement limited enough to make them useful as bioindicators in headwaters surrounding mining claims?

While studies in other systems indicate that sculpins are limited in swimming ability, movement, and home range size (Utzinger et al. 1998, Gray et al. 2004, Natsumeda 2007, Petty and Grossman 2007, Breen et al. 2009), limited studies of Arctic and subarctic sculpin populations suggest possibly longer swimming distances (e.g., Foote and Brown 1998). However, quantitative studies of sculpin movement in these systems are lacking. Consequently, I propose to use Passive Integrated Transponder (PIT) tags and stationary instream readers to measure movement of sculpins during open water months in streams draining the Pebble deposit. I hypothesize that sculpin movement will be limited to short stream reaches (<0.5 km).

Objective 2: What is baseline inter-annual variability in sculpin density, condition, age structure and other factors?

Establishing reference conditions prior to development is essential for maintaining ecological integrity of waters and detecting change that may result from mining. Further, air temperatures in Western Alaska are predicted to increase by 2-3° C and precipitation by 25-50% by the end of the century (Maurer et al. 2007). Data characterizing freshwater habitat in the region of mine claims is limited, making current information unusable to distinguish impacts of mine development from impacts of climate change. Consequently, I propose to establish sculpin biomonitoring sites (approximately 20) both within and outside of mine claims in order to

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characterize baseline inter-annual variability of density and other demographic variables of sculpin populations.

Objective 3: Are hardness-based and/or BLM-generated water quality criteria sufficiently protective for sculpins in waters draining mine claims?

Although large scale mining of numerous claims may generate multiple contaminants, I will focus on copper because the Pebble deposit is primarily a copper deposit (Ghaffari 2011). Copper is toxic to aquatic life, causing acute, chronic and indirect impacts in copper contaminated waters (Clements et al. 1990, Eisler 1998, Sandahl et al. 2007, McIntyre et al. 2012). In salmonids, olfactory receptors are indirectly impacted by increases in copper concentrations of 2-20 µg/L and slimy sculpin may be even more sensitive (Maret and MacCoy 2002, Sandahl et al. 2007, Baldwin et al. 2011). Impaired olfaction can interfere with identification of predator, prey, mates, and kin, ultimately leading to decreased survival.

Copper toxicity varies depending on a number of variables, though is largely influenced by pH, calcium, and DOC (Meyer 2007). The BLM was developed to account for those variables and has been accepted for use by EPA in development of Ambient Water Quality Criteria (EPA 2007). In Alaska, hardness-based copper criteria are used more widely (ADEC 2008). However, recent studies suggest that copper criteria generated by both methods may fail to protect aquatic life in waters draining the Pebble deposit (De Schamphelaere et al. 2004, Welsh et al. 2008, McIntyre et al. 2012, Craven et al. *In prep*). To date, most studies regarding sufficiency of copper criteria have focused on salmonid species, despite indications that sculpins may be both more abundant as well as more sensitive (Adams and Schmetterling 2007). Consequently, I propose to conduct both lab based and field based studies in order to investigate acute, chronic, and indirect effects of copper to sculpins in order to evaluate sufficiency of water quality criteria in waters draining the Pebble deposit.

Lab studies will utilize water derived from the site and/or reconstituted water with similar temperature, pH, calcium, and DOC levels to site water. Sculpins (juveniles and adults) and their eggs collected from headwaters draining the Pebble deposit will be reared in a laboratory environment at variable copper concentrations (2-400 µg/L) in order to determine acute toxicity (LC50); chronic toxicity (EC20) using growth and fecundity as response variables; and indirect impacts to olfactory bulbs as measured by electroencephalogram (EEG, e.g., Hansen et al. 1999) and predation experiments. In the field, marked sculpins will be exposed to variable levels of copper and returned to streams. Subsequently, recapture events will be conducted to measure chronic impacts of copper exposure including growth, condition, and stomach volume. Results of both field and lab components will be used to evaluate copper concentrations at which lethal, sublethal, and behavioral impacts to sculpins occur in site specific waters. Copper concentrations will ultimately be compared to hardness and BLM-based copper criteria in order to evaluate their sufficiency for protecting ecological integrity of Nushagak and Kvichak headwaters. I hypothesize that current copper criteria will prove insufficient, and hope that data gathered in this effort will be useful in considering site specific criteria for discharge should mine development proceed.

EXPECTED RESULTS & CONCLUSIONS

Historically, management and regulatory decisions in salmon ecosystems involved little, if any, baseline or site specific data adequate for measuring impact or conducting restoration efforts, contributing to the worldwide decline of salmon stocks. I expect that sculpins will

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exhibit essential qualities of bioindicator species (Carignan and Villard 2002), and the subsequent establishment of a robust monitoring program using sculpins will assist in preventing and quantifying future impacts to some of the last intact salmon habitats worldwide. Incorporating reference sites into monitoring will assist in distinguishing mining-related impacts from those related to climate change. Further, I expect data collected regarding sculpin sensitivity to copper will inform regulatory decisions regarding discharge of mine waste in Alaska and elsewhere where mine development is rapidly expanding.

BROADER IMPACTS

Tool development: This project will result in information useful to state and federal stakeholders, decision makers, and regulators with respect to mine permitting and development in Alaska and elsewhere. It is intended to inform permitting decisions in a manner that considers site specific information so that mine development and mitigation can best protect ecological integrity of aquatic ecosystems. Robust baseline data can further assist regulators as an 'early warning system' should unintended contamination result from mining. Because so few of Alaska's freshwaters have been evaluated, accurate and detailed taxonomic information for aquatic macroinvertebrates is lacking, complicating the use of macroinvertebrates for biomonitoring. Monitoring sculpins may prove to be a more efficient and accurate tool for assessing impacts to freshwater ecosystems.

<u>Pollution prevention</u>: While the goal of the Clean Water Act is to "restore and maintain the chemical, physical, and biological integrity of the nation's waters by preventing point and nonpoint pollution sources," water quality standards remain chemistry-centric. Although EPA's BLM broadens the consideration of standards to include bioavailability rather than just the aquatic concentration of chemical constituents, it may still fail to protect important aquatic taxa. This proposal will examine the sufficiency of the BLM with respect to the sensitivity to copper of a ubiquitous, immobile species in a region of both rich copper and salmon resources.

Involvement of underrepresented groups: I intend to do my research as part of the University of Washington's Alaska Salmon Program (UW-ASP), which is a research/teaching/outreach program focused on science relevant to understanding and managing salmon-bearing watersheds in western Alaska. For the past decade, UW-ASP has integrated its science and educational activities with two prominent Bristol Bay groups that provide educational opportunities to local residents: the Bristol Bay Native Association and the Bristol Bay Economic Development Corporation. I have worked collaboratively with Nondalton Tribal Council, Bristol Bay Native Corporation, and others independent of UW-ASP for the past several years. Through these collaborations, students and interns from Native Alaskan communities will be integrated with my field research and will obtain broad experience in field sampling methods, data entry, and study design. Additionally, outreach to nearby villages will occur regularly during the course of my research in order to inform local stakeholders about the waters of their region. Finally, when logistically required, field camps will be based out of nearby villages, providing economic benefits to local villagers.

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